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Biogas Production from Co-Digestion of Poultry Manure and Orange Peel through Thermo-Chemical Pre-Treatments in Batch Fermentation

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Abstract— *The increasing demand for the treatment of organic wastes from municipalities, farming and industrial activities is a great opportunity to convert organic wastes into energy in a form of biogas. With the aim of producing biogas from co- digestion of Poultry Manure (PM) and Orange Peel (OP) a series of experiments were carried out for 21 consecutive days. Five different proportions of PM and OP (100%PM, 75%MP+25%OP, 50%PM+50%OP, 25%PM+75%OP, 100%OP) were used to obtain the suitable mix ratio (which gives maximum biogas production). Having determined the optimum mix ratio, chemical pre-treatment with CaO (0.5 g, 1.5 g and 2.5 g) and temperature pre-treatment at 60 and 80 ºC were applied to compare the results with those obtained with non-pre-treated waste. Cumulative biogas production obtained from 75%PM+25%OP was 768ml, whereas 218.33ml was measured from 100%OP. Increasing the proportion of OP above 25% decreased the amount of gas production, volatile solids (VS) and total solids (TS) reduction. This indicated that addition of PM to mix ratios improves biogas production.Thus 75%PM+25%OPmix was found to be the optimum mix ratio which resulted in high biogas yield. In thermal pre-treatments, maximum cumulative gas production was measured at 80 ºC pre-treated substrate. It exceeded by 11.7% and 6.6% over the control and the 60 ºC pre-treated sample respectively. In case of chemical pre-treatments, the highest cumulative biogas yield was obtained from a substrate treated by 2.5g CaO which exceed by 139, 250 and 356 over 1.5g CaO, 0.5g CaO and control, respectively. Overall the results indicated that the biogas yield and VS and TS reduction of the 75%PM+25%OPmix ratio can be enhanced with the use of thermal and chemical pre-treatments prior to anaerobic digestion.*

Keywords—Anaerobic Digestion, Biogas, Co-digestion, Pre-treatments, Total solids, Volatile solids, D-limonene.

I. INTRODUCTION

Biogas technology is growing as a number of countries are accessing up biogas targets as a main approach for treating a variety of organic wastes. Biogas production decreases environmental pollution through decomposing organic wastes and positively impacts the socio-economy of the society (Lawrence, 2012). Today, utilization of biogas as an alternative energy source is steadily increasing. It accounts for up to 20% of renewable energy consumption in the European Union. About 52% of the biogas plants produce biogas from agricultural wastes, and about 36% are utilizing sewage sludge and the remaining 12% are landfill treatment plants. Germany is by far the major biogas producer in the world (Bisypln, 2012).

For their economic progress, African countries need sustainable energy supplies. Unreliable energy supply may end up with low level of private investment in African continent. Therefore, improvement in the quality and magnitude of energy services in developing countries is required to meet developmental objectives including the Millennium Development Goals (MDGs). Although reliable regional energy statistics are not readily available, the existing estimates of energy use in Eastern and Southern Africa indicate that there is a significant and persistent dependence on traditional biomass energy technologies and limited use of modern, sustainable energy technologies (Karekezi, 1994).

Biomass in the form of mainly fuel wood and charcoal is the dominant energy source in Sub-Saharan Africa. Though it appears cheap, overexploitation of this biomass leads to serious negative environmental consequences. Fossil energy sources are the most widely used energy supplies in the world today. However, the increased prices of oil and increased awareness of climate changes is promoting the use of alternative environmentally friendly renewable energy sources such as biogas (Khanal, 2008).

Traditionally, biogas has been used as fuel to support the process temperatures in anaerobic digesters. Another alternative use is that the gas is burned in an engine generator of combustion to produce electricity in biogas plants. It has also been used as fuel for cooking, light and vehicles (Khanal, 2008).

Due to the complex physical and chemical nature of lignocellulosic substrates, their complete biodegradation cannot be achieved in anaerobic digesters to result in high biogas yield (Rafiqu*eet al*, 2010). To overcome biodegradability problem, some pre-treatment methods can be employed (Bruni, 2010). Pre-treatments, for example, biological (Zhong*et al*., 2011), mechanical (Angelidaki and Ahring, 1999), chemical (Devlin *et al.*, 2011),thermal (Mladenovska*et al*., 2006) and combination of these treatments have been done to facilitate the biogas production by overcoming the limitation of hydrolysis, which include the solubilization and biodegradation of hemi-cellulosic and lignin parts of the substrates. Rafique (2010) reported that thermo-chemical pretreatments have a great impact on biogas production with a maximum enhancement of 78% for biogas and 60% for methane. Thermal pretreatment also has effect on biogas production with a maximum enhancement of 28% for biogas and 25% for methane. This indicates that pretreatment of substrates urgently needs further investigation.

Biogas technology was introduced in Ethiopia as early as 1979, when the first batch type digester was constructed at the Ambo Agricultural College. In the last two and half decades around 1000 biogas plants, ranging in size from 2.5m^3 to 200m^3 have beenconstructed in households, community and governmental institutions in various parts of the country (EREDPC, 2008). In Ethiopia, biogas production from different organic materials. However, no research has been done on the effect of different pre-treatments of poultry waste and orange peel on biogas production.

General Objective was to:

- Examine the effect of thermal pre-treatments on biogas production from poultry manure and orange peel in sole or co-digestion.

Specific Objectives were to:

1. Characterize poultry manure and orange peel in terms of the total solids (TS), volatile and fixed solids (VS), moisture content,organic Carbon and pH before and after anaerobic digestion.
2. Evaluate the biogas yield of single and mixed substrates of orange peel and poultry manure.
3. Assess the effect of thermal and chemical pre-treatments on biogas yield

II. MATERIALS AND METHODS

2.1. Description of the Study Area

The study was conducted in Microbiology Laboratory of Haramaya University Main Campus, which is located at a latitude of 9°26’ N and longitude of 42°03’E and has an altitude of 1980 meters above sea level (FAO, 1990).

2.2. Design of Experiments and Preparation of Substrates

The study was carried out by using two experimental phases: (i) anaerobic digestion of five substrates without pre-treatments and (ii) anaerobic digestion of the best performing substrate of first phase with thermal and chemical pre-treatments. The five substrates that were used for anaerobic digestion without pre-treatments were poultry manure (PM) and orange peel (OP) in sole or mixing at different proportions as follows; 100% PM, 75%:25% mix of PM: OP, 50%:50% mix of PM: OP, 25%:75% mix of PM: OP and 100% OP. For further experiment and second phase of experiment the highest biogas yielding substrate was selected and pre-treated by thermal and chemical treatments, i.e. anaerobic digestion after thermal and chemical pre-treatments. The experimental design was completely randomized design. That means the treatments were arranged randomly in the laboratory and done in three replicates.

2.3. Feedstock and Inoculum

Two types of lignocellulosic biomass, poultry manure and orange peel were used in this study. Poultry manure was obtained from Haramaya University animal farm, i.e.fresh manure about (4kg) was randomly collected. Orange peel waste (4kg) was collected from local market around Haramaya Universitywashed with water and cut into pieces using scissors in the laboratory in order to make it easier for digestion. The prepared orange peels were added into poultry manure in different proportions and stored at 4ºC for usage as feed.

To start up anaerobic process, rumen fluid was used as inoculum (Sunarso*et al.*, 2012). For this experiment, fresh rumen fluid was collected from the nearby slaughter house and filtered through a cloth of 0.5mm sieve diameter to separate solid content from slurry. Prior to use, the inoculum was starved for one week by incubating at 38ºCto remove the easily degradable VS present in inoculums (Lo NieeLiew, 2011).

2.4. Analyses of Physico-chemical Characteristics of Substrates

Both poultry manure and orange peel were analysed for TS, VS, moisture content and pH before and after AD process

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based on the Standard Methods for the Examination of Water and Wastewater (APHA, 1999).

2.4.1. Total solids

First a clean evaporating dish was oven-dried (at 105ºC for 1hour), cooled in a desiccator and weighed immediately before use. Sample of substrate (10 g) was placed on the evaporating dish and put in an oven (Contherm 260M) at 105°C using a crucible to evaporate for 24 hours. After 24 hours, the crucible was taken out from the oven, cooled in desiccators and weighed using electronic balance (PB602). Thereafter, the percentage of TS was calculated using the following formula (APHA 2540 B, 1999).

$$\%\mathrm{TS} = \frac{\mathrm{mDS}}{\mathrm{mFS}} \times 100$$

Where,

%TS= percentage of total solids

mDS= mass of dry sample (final weight) in gram

mFS= mass of fresh sample in gram

Then percentage of TS removal was calculated using the formula indicated below.

$$\%\mathrm{TS\ removal} = \frac{\mathrm{TSi} - \mathrm{TSf}}{\mathrm{TSi}} \times 100$$

Where,

Tsi=initial total solids before digestion (%)

Tsf=final total solids after digestion (%)

2.4.2. Volatile and fixed solids

Once the TS was determined, the oven dried sample was ignited at 550°C in a muffle furnace (BiBBY, Stuart) for 3 hours to determine the volatile and fixed solids. The following formula was employed to calculate the percentage of volatile solids content of the TS (APHA 2540 E, 1999).

$$\%\mathrm{VS} = \frac{\mathrm{mDS} - \mathrm{m(ash)}}{\mathrm{mDS}} \times 100$$

Where,

% VS = percentage of volatile solids

mDS= mass of dry solids in gram

m(ash)=remaining mass after ignition =fixed solid in grams.

i.e., TS=VS + fixed solids

Then percentage VS removal was calculated using the equation below.

$$\%\mathrm{VS\ removal} = \frac{\mathrm{VSi} - \mathrm{VSf}}{\mathrm{VSi}} \times 100$$

Where,

Vsi= initial volatile solids before AD (%)

Vsf=final volatile solids after AD (%)

2.4.3. Moisture content determination

To determine the percentage of moisture content (MC) in the samples, 10 g of fresh substrate was dried in an oven (Contherm 260M) at 105 °C for 24 hours and reweighed. The moisture content was then calculated as follows (APHA 2540 E, 1999).

$$\%\mathrm{MC} = \frac{\mathrm{W} - \mathrm{D}}{\mathrm{W}} \times 100$$

Where,

MC = moisture content

W = initial weight of sample in grams,

D = weight of sample after drying at 105 °C in grams

2.4.4. Determination of pH

The initial pH of each sample was measured directly using digital pH meter before and after AD (HANNA HI 8314). In the case of before AD, an electrode was inserted into samples of substrate that was diluted using distilled water before inoculation of rumen fluid and the pH values of the contents of digesters were buffered between 6.8 and 7.4 which is the optimal range for methanogenic bacteria (Arogo *et al.*, 2009). Measurement of pH after AD was also done using pH electrode which was inserted into samples of substrate that is digested in AD process.

2.4.5. Organic carbon

The carbon content of the substrates was obtained from volatile solids data using an empirical equation as reported by Badger *et al.* (1979).

$$\%\mathrm{Carbon} = \frac{\%\mathrm{VS}}{1.8}$$

Where, VS= Volatile solids

2.5. Anaerobic Digestion of Substrates without Pre-treatment

The experiments were conducted in batch mode in 0.5L digester from poultry manure and orange peel which were prepared in five different proportions as indicated above. As suggested by Tchobanoglous*et al*. (1993), substrates were mixed with appropriate amount of distilled water and inoculum to achieve the recommended (8% w/w) total solids content in the fermentation slurry.The total amount of liquid (distilled water and rumen fluid) needed to be added to the digester was then determined by the formula;

$$Y=\frac{mTS-8\%X}{8\%}$$

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Where,
mTS= mass of total solids
X = mass of fresh substrate
Y = mass of fluid (distilled water and rumen fluid) to be added to get 8% total solids in the digester.

Then, by fixing the amount of inoculum (100mL) that was added finally to facilitate digestion, the amount of distilled water that has to be added was then determined using the formula;

Z= Y-100

Where,
Z = amount of distilled water
Y = total amount of liquid (distilled water and rumen fluid).

The temperature of the biodigester was kept at mesophilic condition (38°C) by keeping in oven (Knottier, 2003). The pH of the digesters was maintained between 6.8 and 7.4 by adding buffer solution (Yadvika*et al.,* 2004). The digestion process lasted for about 21 days and biogas yield was measured every day starting from the first day after the substrates were arranged for AD.

2.6. Thermal and Alkali Pre-treatments

For the choice of pre-treatment, the different mix ratios of untreated substrates were identified and compared based on their ability to produce highest yield of biogas production and VS and TS reduction. Therefore, the mix ratio of the untreated substrate that resulted in highest biogas yield was taken as the optimal mix-ratio and used for the next pre-treatment experiments. The pre-treatments were performed before digestion to check whether or not pre-treatments increase the efficiency of biogas production.

2.6.1. Thermal pre-treatment and digestion of high yielding substrate combination

According to Rafique (2010), thermal pretreatment showed enhancement in the temperature range 50-100ºC, with maximum enhancement at 100ºC, having 28% biogas and 25% methane increases. For this reason, the slurry containing the optimum non-treated substrate mix-ratio and the corresponding volume of distilled water were added into 0.5 L flasks. Since temperature below 60ºCis usually considered as a pre-digestion step rather than pre-treatment, 60 and 80 ºC were selected. After covering the flasks with plastic film, they were treated with temperatures of 60 and 80ºC for 3 hours by keeping in water bath with intermittent gentle shaking to ensure the homogeneity of temperatures in the flasks (Bonmati*et al*., 2001). The sample without thermal pre-treatment is used as control. Then all the slurry was kept for 24 hours in a refrigerator at 4ºC before the addition of 100mL inoculum.

The total amount of liquid (distilled water and rumen fluid) needed to be added to the digester was then determined using the same formula indicated in section 3.5 and the same is true for pH and temperature.

2.6.2. Alkali pre-treatment and digestion of high yielding substrate combination

In this study, Slurry containing the optimum non-treated substrate mix-ratio and the corresponding volume of distilled water was added into 0.5 L flasks. Then, different concentrations of CaO (0.5, 1.5 and 2.5 g in solution form) were mixed for 1 hour using rotary shaker. After chemical pre-treatment, the pH of all treatments was reduced to neutral (pH ~7.0) by adding 6N H_2SO_4 (Rafique*et al.,* 2010). After 24 hours of stay in the refrigerator, equal amount of inoculum (100mL) was added to the slurry to adjust the TS to 8%. Digester without CaO addition was used as a control.

The total amount of liquid and amount of distilled water was calculated as mentioned above, and same is true for temperature and pH of the slurry.

2.7. Digester Configuration and Setup for Biogas Production

Thirty (15 for co-digestion without pre-treatment, 9 for chemical pre-treatment and 6 for thermal pre-treatment) anaerobic digesters (plastic bottle) were constructed for bench-scale experiments with which biogas was produced out of the degradation of substrates in 0.5L digester. Degradation of the substrate was accomplished in sealed three bottles each with a capacity of 0.5L which were arranged in order in such a way that the first bottle contained slurry, the middle contained acidified brine solution and the last was used for collecting the brine solution that was expelled out from the second container.

The acidified brine solution was prepared by adding NaCl to distilled water until a supersaturated solution was formed to prevent the dissolution of biogas in the water. Three drops of sulphuric acid were added using a dropper to acidify the brine solution. All the three containers were interconnected with a plastic tube having a diameter of 1cm. The tube connecting the first bottle to the second was fitted just above the slurry in the first bottle to help gas collection. Thus, the biogas produced by fermentation of the slurry was driven from the first bottle to the second bottle that contained a brine solution so as to displace a volume of the

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brine solution equivalent to the volume of biogas that was produced.

The lids of all digesters were sealed tightly using superglue in order to control the entry of oxygen and loss of biogas. Daily biogas production was measured following the method suggested by Itodo*et al* (1992). As biogas production was commenced in the fermentation chamber, it was delivered to the second chamber which contained the acidified brine solution. Since the biogas is insoluble in the solution, a pressure build-up provides the driving force for displacement of the solution. The displaced solution was measured to represent the amount of biogas produced. The temperature of all digesters was maintained at 38°C by keeping in an incubator, which represents mesophilic condition.

2.8. Data Analysis

Data were analysed by using analysis of variance (one-way ANOVA) using SAS version 9.1. Fishers Least Significant Difference (LSD) was used to investigate statistical significance between the different treatments, whereas paired samples T-test was used to investigate statistical significance within a treatment. The statistical significance level was selected at p-value < 0.05

III. RESULTS AND DISCUSSION

3.1. Physico-chemical Characteristics of the Untreated Substrates

The Physico-chemical characteristics of both PM and OP in sole or mixed for AD were determined before and after AD, and among the different mix ratios.

Table.1: Comparison of pH, % organic carbon and %MC between before and after AD and among different mix ratios (values are mean ± SE, n=3)

Treatments	Parameters					
	pH		% C		%MC	
	Initial	Final	Initial	Final	Initial	Final
A	6.89±0.01^{Aa}	8.15±0.01^{Eb}	10.14±0.01^{Da}	8.52±0.01^{Db}	76.40±0.03^{Ea}	80.60±0.05^{Eb}
B	6.82±0.00^{Ba}	8.46±0.02^{Db}	10.69±0.01^{Ea}	7.68±0.00^{Eb}	76.20±0.06^{Da}	82.20±0.03^{Db}
C	6.51±0.08^{Ca}	8.64±0.02^{Cb}	11.36±0.01^{Ca}	8.87±0.01^{Cb}	74.90±0.03^{Ba}	78.80±0.01^{Cb}
D	6.13±0.04^{Da}	8.73±0.06^{Bb}	11.74±0.02^{Ba}	9.19±0.02^{Bb}	74.10±0.02^{Ca}	77.40±0.05^{Bb}
E	5.53±0.02^{Ea}	8.83±0.03^{Ab}	12.02±0.01^{Aa}	9.80±0.01^{Ab}	73.35±0.04^{Aa}	77.00±0.06^{Ab}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.

A=100%PM, B=75%PM+25%OP, C=50%PM+50%OP, D =25%PM+75%OP and E=100%OP.

pH is one of the factors that affect anaerobic digestion. It is important to adjust the pH-value in the optimal range because anaerobic performance is affected by slight pH deviations from the optimum. A significant decrease in growth rate of methane forming bacteria occurs if the value of pH is below 6.6. Furthermore, high alkaline pH can cause disintegration of microbial granules and consequently, result in the failure of anaerobic digestion (Ward, 2008). The pH of 100% PM slurry before anaerobic digestion was about 6.89±0.01, whereas that of 100% OP was 5.53±0.02. So, pH of poultry manure alone is almost optimal for biogas production, but pH of OP alone is not optimal for anaerobic digestion as it falls below 6.8. When the substrates were mixed, it resulted in the rise of pH compared to that of OP alone. The pH was found to increase significantly with increasing of PM proportion in the mix, suggesting that PM helps to maintain the pH to meet the optimum required. As volatile acid concentrations increase, the pH in the digester decreases. Thus, mixing of substrates is a good way of adjusting the pH value to the optimum (Hills and Roberts, 1981).

Comparison of pH values between before and after AD showed that pH values are significantly increased for all treatments (P<0.05) (Table 1). Maximum pH value was 8.83 whereas minimum value was 8.15. This indicated that as the proportion of OP increased within the sample, pH value also increased accordingly (Table 1). The reason for the increment of the pH values after AD may be attributed to production of alkali compounds, such as ammonium ions during the degradation of organic compounds in the digester (Gerardi, 2003). The pH value of the rumen fluid used in all experiments was relatively higher than both substrates (pH=7.51). This shows that the rumen content used may have high ammonia concentration. Thus, in addition to initiating the start up in the digestion process, the rumen

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fluids were used to adjust the pH of both single and mixed substrates, especially OP alone and mix ratios containing high content of OP.

The moisture content of 100% PM, 75% PM+25% OP, 50% PM+50% OP, 25% PM+75% OP and 100% OP before AD were 76.40±0.03%, 76.20±0.06%, 74.90±0.03%, 74.10±0.02%, and 73.35±0.04%, respectively. This indicates that PM contains high moisture content than OP and mixing of substrates might balance the moisture content of the digester. Significant differences were observed between before and after AD in all treatments (Paired samples T-test, $P<0.05$). The moisture content in all the substrates was found to be high to facilitate efficient degradation of the substrates as bacteria can easily access liquid substrate for relevant reactions to take place easily (Buysman, 2010). Since studies on the most favourable percentage of total solids for biogas productions suggest 8% as the optimum TS, the initial moisture content of substrates used for this study was not optimal for wet anaerobic digestion process (Tchobanoglous*et al*., 1993). Therefore, dilution is required to bring the total solids percentage to 8%.

There was a significant difference between treatments in both before and after AD in %C (Table 1). The study revealed that the percentage degradation of organic carbon for 75% PM+25% OP was higher than all treatments (from 10.69±0.01to 7.68±0.00, i.e., 30.1% reduction) (Table 1). Organic carbon can be removed in anaerobic digesters either by being converted to cellular materials for growth and reproduction of bacteria or through biogas production (Gerardi, 2003). Therefore, the decrease in Carbon reflects the degradation process during anaerobic digestion (Devlin *et al*, 2011). The results also revealed that there were differences in percentage organic carbon in all mix ratios before and after AD (P<0.05). This shows that mixing balances the percentage of organic carbon of substrates in the digester as the two substrates (PM and OP) contain different carbon content.

3.2. Analysis of TS and VS values of Untreated Substrates before and after AD

Table.2: Comparison of TS % and VS % between before and after AD and among the mix ratios (values are mean ± SE, n=3).

	Parameters			
	Initial TS	Final TS	Initial VS	Final VS
100% PM	23.55 ± 0.02^{Db}	19.44 ± 0.02^{Da}	18.25 ± 0.02^{Da}	15.34 ± 0.02^{Db}
75% PM+25% OP	23.82 ± 0.34^{Db}	17.75 ± 0.02^{Ea}	19.24 ± 0.02^{Ea}	13.82 ± 0.01^{Eb}
50% PM+50% OP	25.07 ± 0.03^{Cb}	21.24 ± 0.02^{Ca}	20.45 ± 0.02^{Ca}	15.96 ± 0.01^{Ca}
25% PM+75% OP	25.93 ± 0.03^{Bb}	22.64 ± 0.01^{Ba}	21.13 ± 0.03^{Ba}	16.54 ± 0.02^{Bb}
100% OP	26.45 ± 0.02^{Ab}	23.04 ± 0.01^{Aa}	21.64 ± 0.01^{Aa}	17.64 ± 0.01^{Ab}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments. PM= poultry manure, OP= orange peel.

Significant differences were observed between treatments in % TS and %VS both in before and after AD (Table 2). Total solid content of all mixes before inoculation and digestion fall between 23.55±0.02% (i.e., 2.36 gram of TS from 10-gram sample) and 26.45±0.02%. Maximum TS was measured from 100%OP, but the minimum TS was recorded from 100%PM as shown in the table above (Table 2). The TS content of 23.55% of PM used in this experiment is in the range of 10 to 30% TS reported by Braun (1982). Some agro-industrial wastes may contain less than 1 % TS, while others contain high TS content of more than 20 %. Thus, the TS content of OP alone was in this range. This results in some substrates being able to be fermented only when mixed with other substrate or diluted.

After AD, values of TS significantly decreased in all substrate types (Table 2). However, high decrement was observed in 75%PM+25%OP which was 6.07. The result also revealed that significant differences were observed between substrates in VS before and after AD. High reduction of VS was measured in 75%PM + 75%OP mix substrates compared to the rest of substrates after AD (Table 2). The TS and VS values before digestion was found to vary significantly ($P<0.05$) with increasing of OP proportion in the mix, suggesting that mixing helps to adjust the TS and VS. Removal of VS after AD suggests its conversion to biogas. Total solids and volatile solids destruction is a good parameter for evaluating the efficiency of anaerobic digestion (Abuabaker and Ismail, 2012).

3.3. Average Daily and Cumulative Biogas Production of Untreated Substrates

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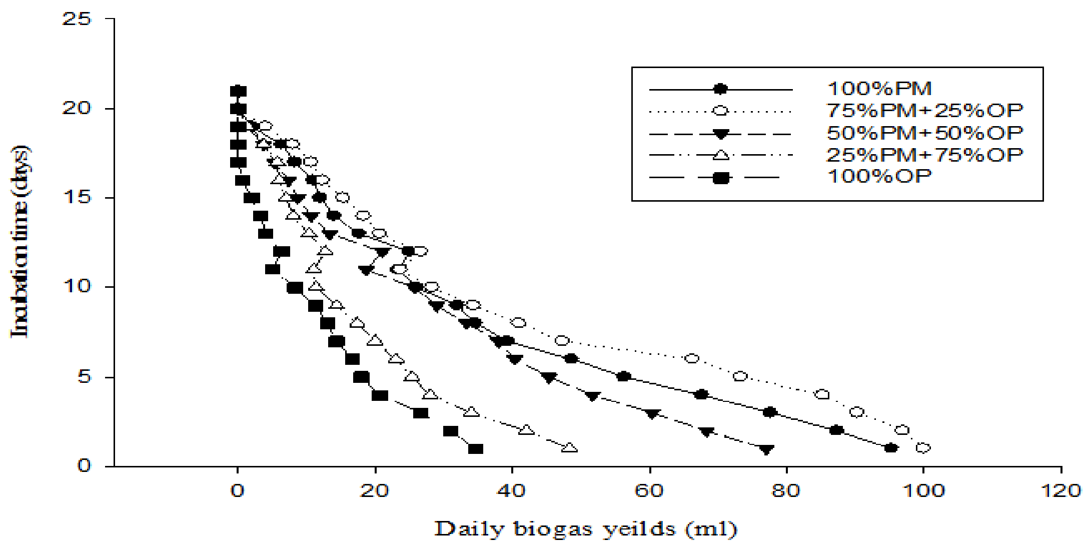
Even though the digesters contained different mix ratios of PM and OP, and the volume biogas produced varied with substrate mixture, gas production was noticed from the very initial day of the experiment (Figure 1, Appendix Table 1). Initially, the digester with PM alone and 75% PM + 25%OP produced higher amount of biogas than other digesters (Figure 1). This could happen due to the presence of higher amount of readily biodegradable organic matter and native anaerobic microbes in the PM (Hobson, 1981; Yeole and Ranande, 1992). Thus, biogas production is a function of the feedstock's organic content and its biodegradability (Macias-Corral *et al*., 2008).

Production of gas had gradually decreased starting from the first day in all digesters except in PM alone. This might be due to the declining of readily decomposable substrate (Ahn*et al*., 2009) and/or an increase in ammonium concentration that may resulted in an increased pH values (Hansen *et al.*, 1999). Gas production continued until day 19 and fallen sharply to 0ml after day 20 for digesters having PM alone and PM as co-substrate, but it stopped after day 17 for digester containing OP alone.



PM=Poultry manure, OP= Orange peel

Fig.1: Daily mean biogas yield of the different substrate combinations.

There was a significant difference between the substrates in an overall biogas yield (Figure 2, $p<0.05$) even though closer result was obtained from 100%PM and 50%PM+50%OP. High production of gas was recorded from a digester containing PM alone and other digesters having equal or more than 50% of PM as a co-substrate (Figure 2). However, the highest production of gas was observed from the mix ratio of 75% PM + 25% OP. From 10g (75%PM+25%OP), 768ml (Appendix table 1) of biogas was produced which was 549ml higher than 100%OP, that has produced 218.33ml of cumulative biogas. According to Kapraju and Rintala (2006) the performance of digesters could be considerably improved by means of co-substrate addition and hence can be used to increase the efficiency of degradation and biogas production.

Low gas production obtained from digesters having high proportion of OP and the lowest production of gas was measured from OP alone. This may be due to the presence of an antimicrobial compound 'D-Limonene' in OP (Martin *et al.*, 2010). This chemical constitutes 90% of oranges essential oil as 2-3% of dry matter of the orange (Mizuki*et al.*, 1990). Limonene has been reported to be highly toxic to anaerobic digestion (Martin *et al.*, 2010). It causes ultimate failure of the process at concentration of 400 μL/L on mesophilic digestion (Mizuki*et al.*, 1990) and in the range of 450 to 900 μL/L on thermophilic digestion (Forgacs, 2012). Thus, it can be concluded that co-digestion of PM and OP is more productive with OP proportion not exceeding 25%. The higher production from the mixtures could be due to a proper nutrient balance, increased buffering capacity, and decreased effect of toxic compounds resulting from mixing of substrates (Fulford, 1988; Macias-Corral *et al*., 2008; Li *et al.*, 2009; Tamirat, 2012).

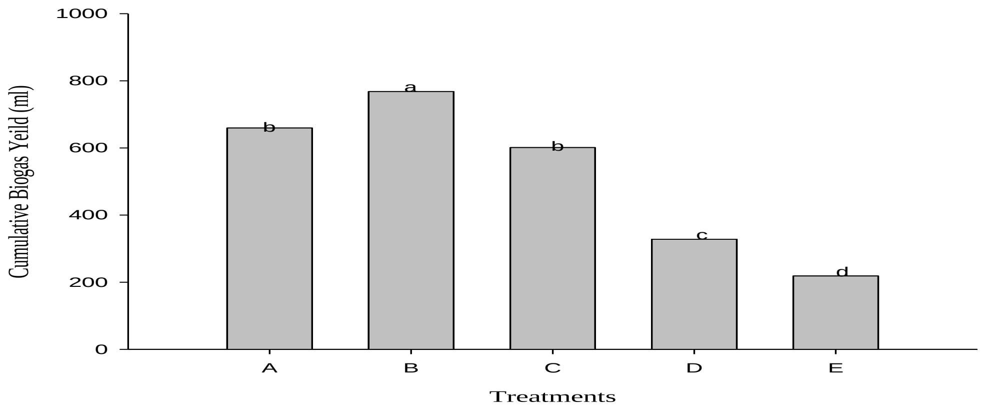


Fig.2: Cumulative biogas yield of the different substrate combinations (Values are mean ± SE). Bars with different letters indicate significant differences between means while those with same letters show no significant difference between means. A=100%PM, B=75%PM+25%OP, C=50%PM+50%OP, D=25%PM+75%OP, E=100%OP. (PM=Poultry manure, OP=Orange peel)

3.4. Physico-chemical Characteristics of Temperature Pre-treated Substrate

pH values of the substrates for the three temperature treatments (control, 60 ºC and 80 ºC) was within the range of 6.82±0.01 to 7.43±0.01 before digestion (Table 3). This pH range is optimal for biogas production. Optimal pH for biogas production is neutral and when pH is < 6 or >8, fermentation process will be inhibited or ceased at all because of its toxic effect on the methanogenic bacteria, which produce methane gas (Thy *et al*., 2003). The pH value of rumen fluid used in this experiment is almost neutral (7.51).

Table.3: Physico-chemical features of blended PM and OP at 75%: 25% ratio for thermal pre-treatment test before and after AD (values are mean ± SE, n=3).

Treatments	Parameters			
	Initial pH	Final pH	% initial organic C	% final organic C
Control	6.82 ± 0.01^{bA}	8.45 ± 0.01^{aA}	10.69 ± 0.01^{aA}	7.68 ± 0.01^{bA}
60 ºC	7.41 ± 0.01^{bB}	8.45 ± 0.01^{aA}	10.56 ± 0.05^{aA}	3.65 ± 0.02^{bB}
80 ºC	7.43 ± 0.01^{bB}	8.46 ± 0.01^{aA}	10.59 ± 0.03^{aA}	3.17 ± 0.01^{bC}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.

The result showed that the values of pH were slightly increased as the temperature of the substrate rose up from control to 80ºC. This may be explained by the solubilisation of compounds such as proteins during thermal pre-treatment (Carrère*et al.*, 2009). So this indicates that temperature and pH are directly proportional to each other, i.e., as temperature increases pH increases and vice versa up to a certain point. There was no significant difference in pH values between the thermal treatments after AD (*P*>0.05) (Table 3). Before AD, pH value of the control showed significant difference than the two thermal treatments compared to initial pH (p<0.05). The final alkaline pH observed after digestion might be explained by the formation of $(NH_4)_2CO_3$ (Georgacakis*et al.*, 1982).

The result revealed that %C reduced in both thermal treatments (60ºC and 80ºC) before AD. The percentage reduction was 65.4% and 70% for 60ºC and 80ºC respectively. The results also showed that there are significant differences in percentage organic carbon in all treatment before and after digestion (*p*<0.05). The maximum reduction of carbon content observed in 80 ºC thermal treatment (exceeded by 41.8% over the control) might be due to either by being converted to cellular materials for growth and reproduction of bacteria or biogas production (Gerardi, 2003). As reported by Abdel-Hadi and

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El-Azeem (2008) the decrement of organic C indicates the effectiveness of degradation process during anaerobic digestion.

3.5. Effect of Thermal Pre-treatments on TS and VS Reduction

As shown in (Figure 3), there was no significant difference in TS between thermal treatments before digestion even though significant difference was observed after AD. TS of the substrates pre-treated by 60ºC and 80ºC temperature following digestion were significantly lower than the control (22ºC), although there was no significant difference between 60ºC and 80ºC pre-treatments (Figure 3). This reflects that increment of temperature of pre-treatment may reduce the TS value of substrate after AD and result in increased biogas production. TS was significantly reduced within each thermal treatment after digestion. This decrement in TS demonstrates that a large fraction of the substrates was broken down and digested. During anaerobic digestion, the TS of the substrate decreased due to its consumption for biogas production (Gerardi, 2003).

The initial value of TS showed that the moisture content of the substrates to be only 76.2%. Since studies on the most favourable percentage of total solids for biogas productions suggest 8% as the optimum TS, the initial moisture content of substrates used for this study was not optimal for wet anaerobic digestion process (Tchobanoglous*et al*., 1993). Therefore, 119.75 mL (100 mL inoculum+19.75 mL distilled water) is required to bring the total solids percentage to 8%.

There was no significant difference between temperature treatments in VS before AD. However, significant difference was measured in VS between treatments after AD (Figure 6). That is, VS of the substrates pre-treated by 60 and 80ºC temperature following AD was significantly lower than that of control temperature, though there was no significant difference between 60 and 80 pre-treatments (Figure 4). Percentage reduction of VS for control, 60 ºC and 80 ºC pre-treated feed stocks were 28.2%, 61.1% and 64.3%, respectively. The observed volatile solid reduction could be due to an increment of soluble materials (Ferrer *et al*., 2008), due to thermal pre-treatment, which increases the availability of substrate for microbes during anaerobic digestion (Carrère*et al*., 2009).

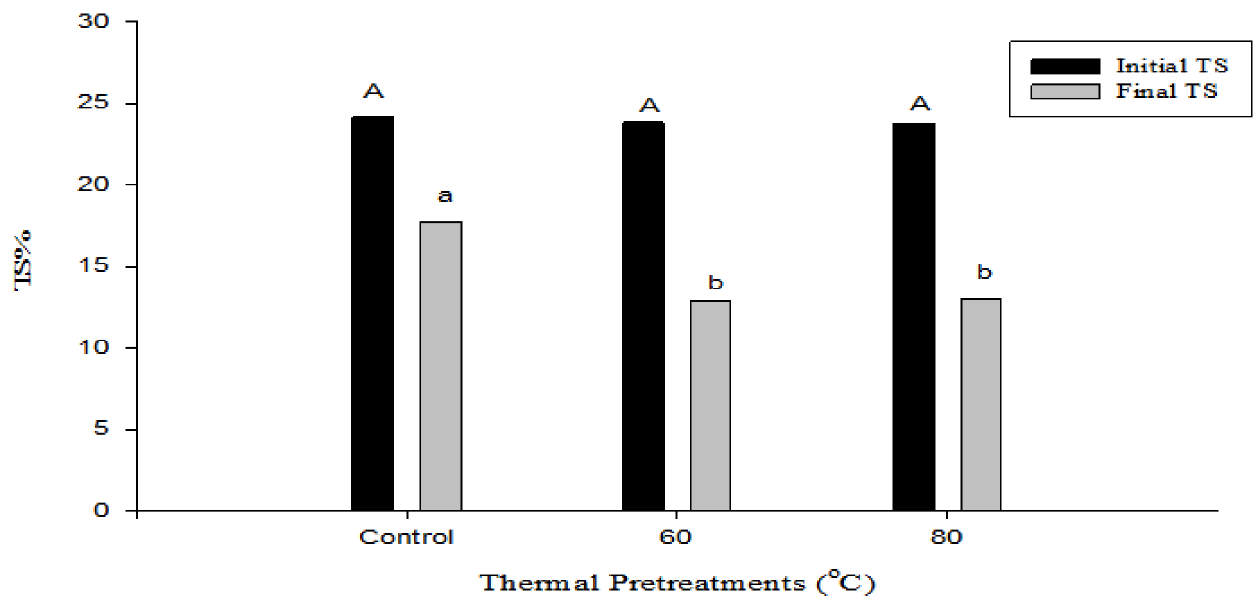

Fig.3: Values of TS for thermally pre-treated substrates before and after digestion. Capital letters represent differences between %TS of the substrate under different temperature pre-treatments before digestion while small letters represent that of after digestion. Bar graphs with the same capital or small letters are not significantly different, whereas those with different capital or small letters are significantly different. TS=Total Solids.

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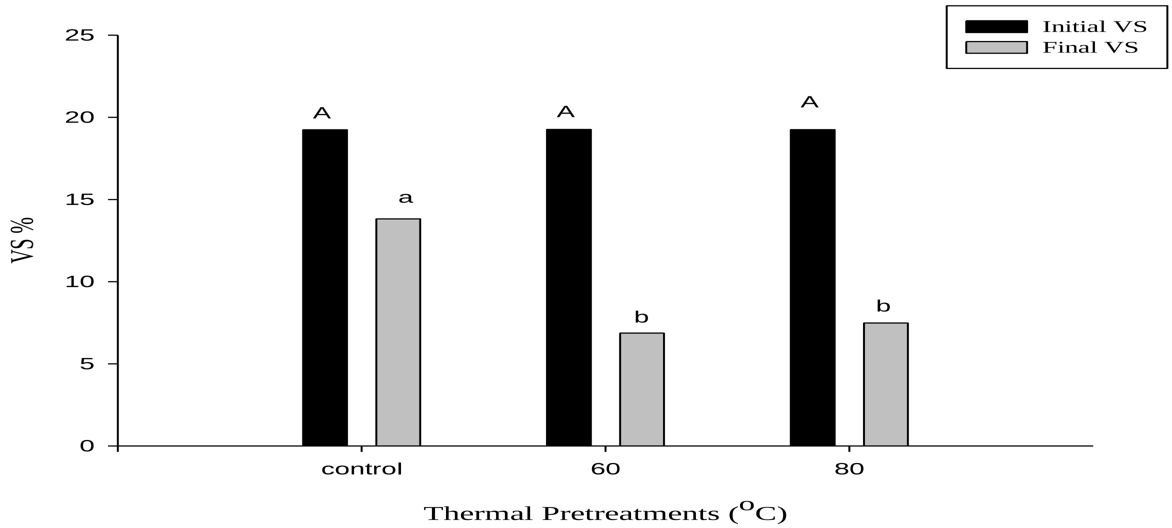

Fig.4: Values of VS for thermally pre-treated substrates before and after digestion. Capital letters represent differences between %VS of the substrate under different temperature pre-treatments before digestion while small letters represent that of after digestion. Bar graphs with the same capital or small letters are not significantly different, whereas those with different capital or small letters are significantly different. VS=Volatile Solids.

3.6. Biogas Production from Thermally Pre-treated Substrates

The average biogas production of control and 60ºC was almost closer to each other at day 1 and 2 even though the production was higher in case of the substrate pre-treated by 80ºC (Figure 5). After day 3 production of biogas from control was less than those obtained from both thermally pre-treated substrates. This illustrates that the substrate treated by 80ºC is easily digestible by bacteria that take part in anaerobic digestion particularly hydrolytic bacteria at the early stage of the digestion. The production of gas gradually decreased from day 1 to day 17 and completely stopped starting from day 18 in all digesters containing thermally pre-treated substrate. Thus, pre-treatment does not only yield greater amount of biogas, but it also reduces hydraulic retention time needed for AD (Ferrer *et al*., 2008).

For thermally (60 and 80 ºC) pre-treated samples more than 50% of biogas were measured within 5 days. This indicates that availability of more easily degradable organic materials for microbes within this short period of time. The increased initial biogas production is credited to the increased accessibility and degradability of substrate (Rafique*et al*., 2010).

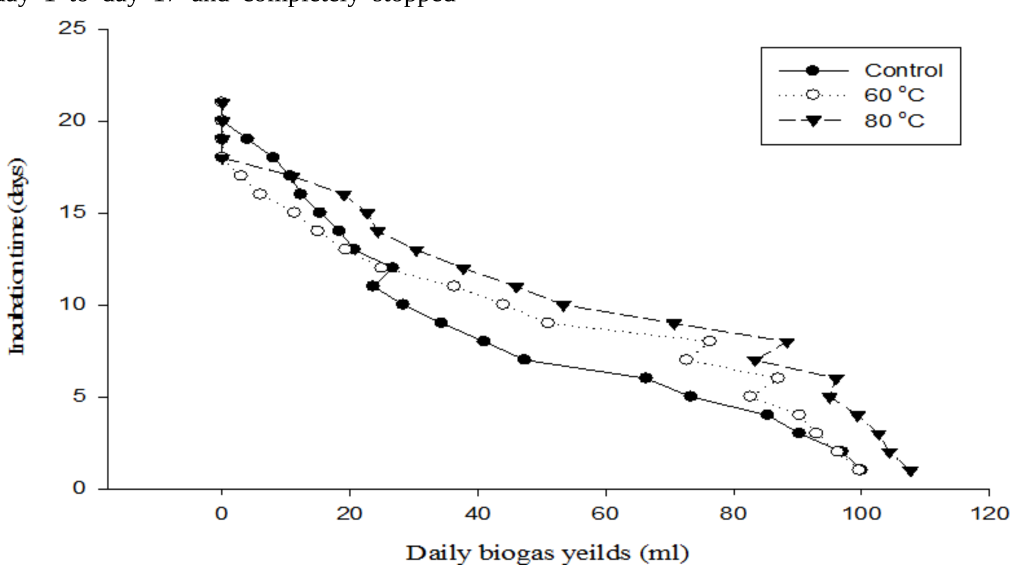

Fig.5: Daily mean biogas yield profile during batch fermentation of thermally pre-treated substrates.

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Substrates pre-treated with 60 or 80 ºC significantly increased cumulative biogas yield when compared with the control temperature (*P*<0.05, Figure 6). The result also revealed that there was significant difference between 60 and 80 ºC treated substrate in cumulative biogas yield (*P*<0.05). Maximum cumulative gas production was measured for 80 ºC pre-treated substrate. It was exceeded by 11.7% over the control and 6.6% over 60 ºC pre-treated sample.

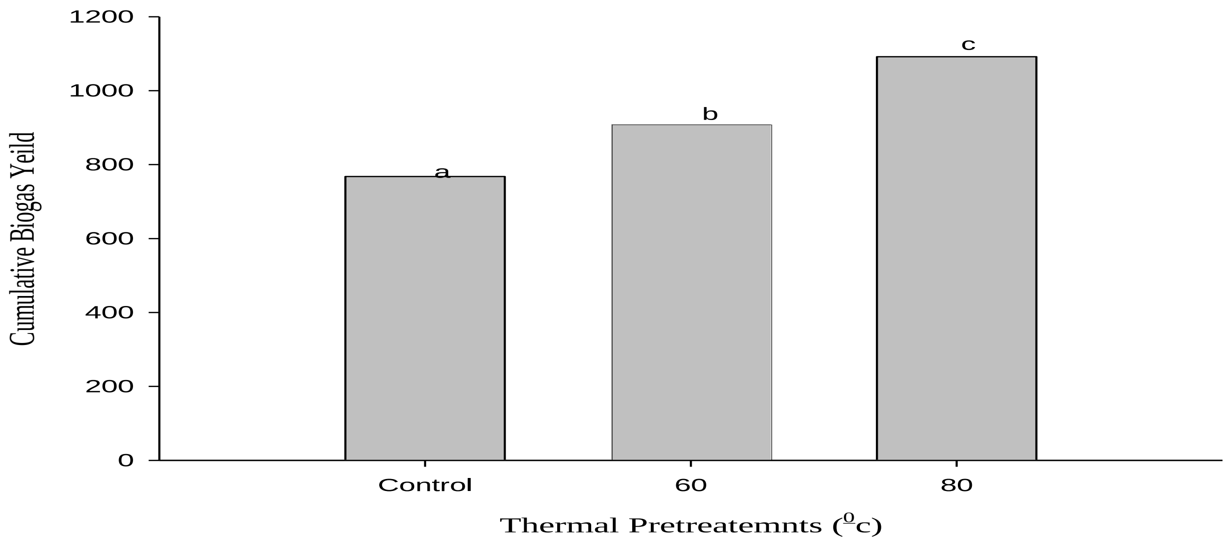

Fig.6: Cumulative biogas yield of the different level of thermally pre-treated substrates (means with the same letter are not significantly different.

3.4. Analysis of pH and %C of Alkali Pre-treated Substrate

Table.4: Characteristics of blended PM and OP at 75%: 25% ratio for chemical pre-treatment before and after AD (values are mean ± SE, n=3).

Amount of CaO(g)	Parameters			
	Initial pH	Final pH	% initial organic C	% final organic C
0 (Control)	6.82 ± 0.01^{bC}	8.45 ± 0.01^{aA}	10.69 ± 0.01^{aA}	7.68 ± 0.01^{bA}
0.5	7.49 ± 0.01^{bB}	8.12 ± 0.03^{aA}	10.6 ± 0.01^{aA}	4.93 ± 0.34^{bB}
1.5	7.51 ± 0.01^{bB}	8.46 ± 0.01^{aA}	10.59 ± 0.01^{aA}	4.02 ± 0.32^{bC}
2.5	O7.54 ± 0.00^{bA}	8.45 ± 0.02^{aA}	10.57 ± 0.01^{aA}	2.98 ± 0.01^{bD}

Means followed by different small letters in row are significant at 0.05 probability levels for paired samples T-test within treatment. Means followed by different capital letter in column are significantly different at 5% level of significance between treatments.

The initial pH value of all the substrate was in the range of 6.82±0.01to 7.54±0.05, which is ideal for anaerobic digestion (Thy *et al*., 2003). Samples treated with CaO were buffered by 6 H_2SO_4 in order to keep the pH of the digester around neutral. This speeds up the activity of microbes which were involved in AD as they are sensitive to pH. After digestion, no significant difference was observed between the treatments, and all of them were existed within the range of alkaline pH. This may be attributed to the formation of $(NH_4)_2CO_3$ after digestion (Georgacakis*et al.*, 1982) (Table 4). Significant %C variation was observed before digestion between control and chemically treated substrates even though no significant difference was seen among chemically treated substrates. In all treatments significant difference in %C was observed after AD (p<0.05 Table, 4.4). Percentage of carbon degradation of control, 0.5g CaO, 1.5g CaO and 2.5g CaO were 28%, 53%, 62% and 71%, respectively. This indicates that degradation of organic carbon increased with increment of Cao concentration to some extent. The increase in the C degradation demonstrates the effectiveness of digestion process for pre-treated substrate as organic carbon is removed in anaerobic digesters through its conversion either into gas or cellular materials (Abdel-Hadi and El-Azeem, 2008).

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3.8. Analysis of Total Solids and Volatile Solids Reduction.

Before AD, no significant difference was seen among chemically treated substrates in TS, and between chemically treated substrate and the control. However, variation was noticed in all treatments after digestion (Figure 7). The amount of TS was slightly decreased as concentration of CaO from 0 to 2.5g. This shows that amount of CaO added to the substrate affects TS value of the substrate. Percentage reduction of TS of a control, and the samples treated by 0.5g, 1.5g and 2.5g of CaO were 25.48%, and 28.9%, 36.35% and 44.66%, respectively. That is, %TS of the substrates treated with the highest amount of CaO was significantly lower than all the other treatments after AD. Comparison of TS for each chemical treatment between before and after digestion showed that TS was significantly reduced after digestion due to its consumption to biogas (Gerardi, 2003).

Initially, the percentage total solids of the substrate show that the moisture content of the substrate to be only 76.2%. Since studies on the most favourable percentage of total solids for biogas productions suggest 8% as the optimum TS, the initial moisture content of substrates used for this study was not optimal for wet anaerobic digestion process (Tchobanoglous*et al*., 1993). Thus, considering 100 mL of inoculums added, an additional 19.75 mL of water is required to bring the total solids 8%.

There was no significant difference in %VS among chemically treated substrate before AD, but high reduction of VS was observed in all treatments after AD (Figure 8). Percentage reduction of VS in control was 28.25%. However, high reduction was observed in substrate pre-treated by CaO compared to control. The reductions were 54.64%, 63.41% and 73.67% for substrates received 0.5g, 1.5g and 2.5g of CaO, respectively. This illustrates that as amount of CaO (0 to 2.5g) added to the substrate increased, %VS increased. This could be resulted from the exposure of biodegradable matter previously unavailable to microorganisms and from the alteration of the composition of hardly degradable compounds (Carlsson*et al*., 2012).

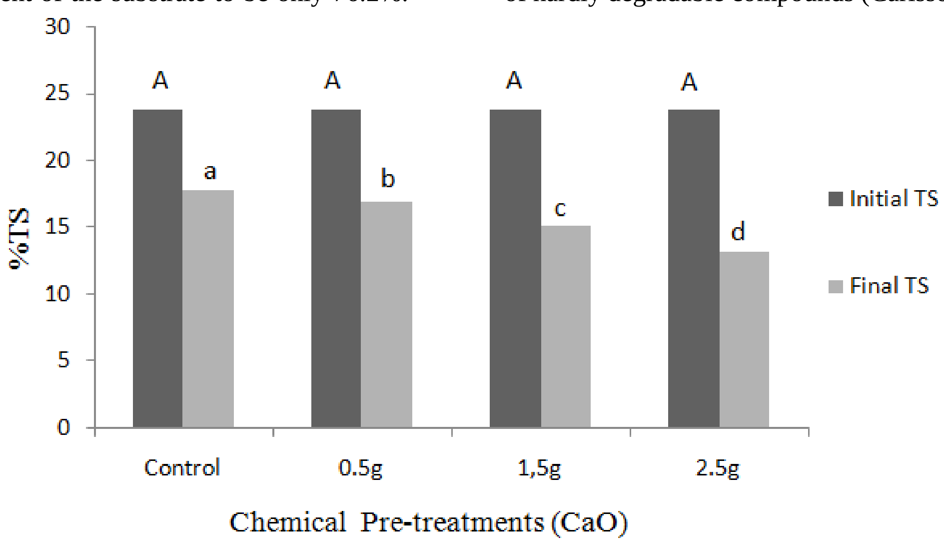

Fig.7: Values of TS for CaO pre-treated substrates before and after digestion. Capital letters represent differences between %TS of the substrate under different chemical pre-treatments before digestion while small letters represent that of after digestion. Bar graphs with the same capital or small letters are not significantly different, whereas those with different capital or small letters aresignificantly different. TS=Total Solids

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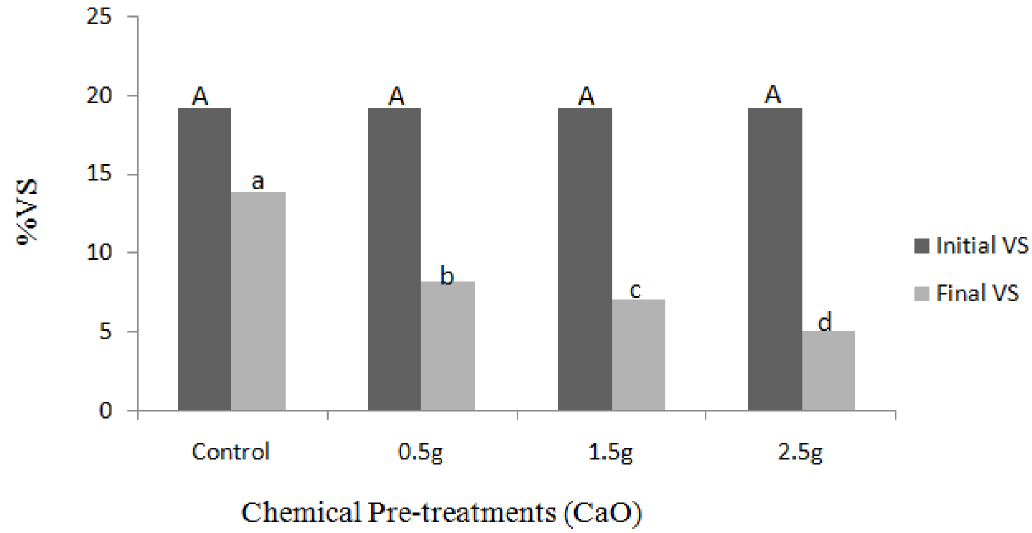

Fig.8: Values of VS for CaO pre-treated substrates before and after digestion. Capital letters represent differences between %VS of the substrate under different chemical pre-treatments before digestion while small letters represent that of after digestion. Bar graphs with the same capital or small letters are not significantly different, whereas those with different capital or small letters are significantly different. VS=Volatile solids

3.9. Biogas Production of Alkali Pre-treated substrates

Biogas produced from all digesters was recorded from day 1 to day 21 (Appendix table 3). Gas production from a substrate treated by 1.5g and 2.5g of CaO was 0 until the second day. This could be due to the addition of 6N H_2SO_4 to maintain the pH at neutral. Addition of sulphuric acid results in by-products such as 5-hydroxylmethylfurfural (5-HMF)and furfural (Larsson *et al*., 1999). Though these by-products do not inhibit methane production from xylose (Barakat*et al*., 2012), the methanogenic microorganisms require a period of adaptation to start methane production.

In a substrate which received 0.5g of CaO, gas production was started at day 2 because of low concentration of CaO, and this might not affect the activity of microbes. Maximum production of gas was observed at day 4 from all chemically pre-treated samples as microbes effectively degrade the substrate. After day 4, production of gas from chemically subjected substrates was slightly decreased and finally ceased at day 18 although some fluctuations were noticed a few days (Figure 9). This could be attributed to scarcity of the necessary nutrients from the digesters (Hansen *et al*., 1998).

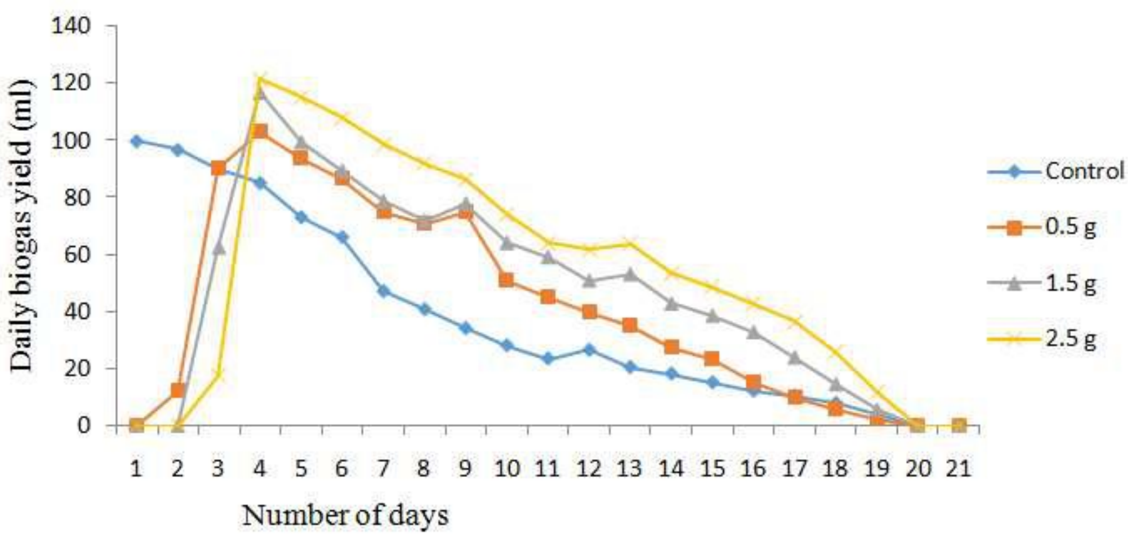

Fig.9: Daily mean biogas yield profile during batch fermentation of chemically pre-treated substrate

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Among all treatments, significant difference in gas production was noticed even though high variation was not observed between control and a sample treated by 0.5g of CaO (Figure 10). The highest cumulative biogas production was obtained from a substrate treated by 2.5g CaO which exceed by 139, 250 and 356 over 1.5g CaO, 0.5g CaO and control, respectively. Compared to the other chemically treated substrates, the lowest gas yield was obtained from a sample treated with 0.5g of CaO.

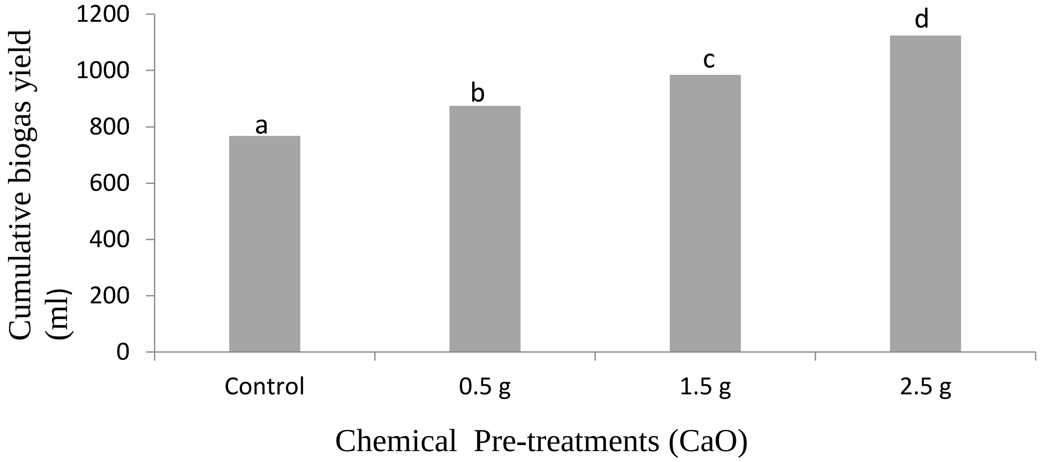

Fig.10: Cumulative biogas yield for the different level of CaO pre-treatments (means with the same letter are not significantly different).

IV. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

4.1. Summary and Conclusions

The main aim of the study was to produce biogas from co-fermentation of poultry manure and orange peel at different mix ratio which took place under a series of experiments at mesophilic condition. This series of experiments were taken place within 21 days. Anaerobic digestibility tests were carried out to get appropriate mix ratio for maximum biogas production from wet co-digestion of PM and OP at 5 different proportions. During the first phase of experiments, the mix ratio of 75%PM+25%OP resulted highest biogas yield compared to the rests, and selected for second phase of experiment. Then 75%PM+25%OP was pre-treated with temperature of 60°C and 80°C and different concentrations of CaO(0.5g, 1.5g and 2.5g) in order to identify the effect of pre-treatments on biogas production.

Thermal pre-treatments were carried out by treating the selected best performing substrate (75%PM+25%OP) with temperature of 60°C and 80°C. Maximum production of biogas was obtained from a mix ratio treated by 80°C compared to control and a sample treated by 60°C. Cumulative biogas production from a sample treated with 80°C was 1091.67ml, while it was 768ml and 909ml from control and a substrate treated by 60°C respectively. Maximum reduction of TS and VS, and high degradation of organic carbon was noticed in a mix ratio subjected by 80°C. Chemical pre-treatment was done by treating 75%PM+25%OP mix ratio with different amount of CaO (0.5g, 1.5g and 2.5g). It was noticed that cumulative biogas yield from CaO pre-treated substrate increase gradually with gram of amount of CaO added, indicating that more organic material was available for microbes for degradation. Significant difference of gas production was observed between the treatments ($p>0.05$). The maximum production of biogas (1124ml) was obtained from a sample pre-treated by 2.5g of CaO. This may be due to the increment of degradability of substrate after pre-treatments. This in turn leads to high availability of nutrients for microbes, and finally improves biogas production. Generally, pre-treatments modify biogas production from different feed stocks as they speed up the activity of microbes.

4.2. Recommendations

Based on the finding of the study, the following recommendations are given;

- ✓ Ranking of mix ratios should be done based on reduction of TS, VS, organic Carbon, and producing the highest biogas yield in order to select a mix ratio for Thermal and Alkali pre-treatments.
- ✓ Other combination effective pre-treatments could be used to identify the most relevant one which improve the production of gas without eradicating the nutrients of the feed stocks, and initiating the activity of microbes.
- ✓ The five mix ratios could be characterized based on organic loading rate, Carbon/Nitrogen ratio and Carbon/ Phosphorous ratio to assess their effect on biogas production.

- ✓ Orange peel should be pre-treated by appropriate pre-treatments to reduce the inhibitory effects and optimize biogas production.

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APPENDICES

Appendix Table 1. Daily mean biogas yields from co-digestion ± SE (mL) (n=3)

	Mix ratio				
Days	100% PM	75%PM+25%OP	50%PM+50%OP	25% PM+75%OP	100% OP
1	95.33±0.88	100±1.15	77±1.15	48.33±1.20	34.67±1.20
2	87.33±0.88	97±1.15	68.33±0.88	42±1.15	31±0.57
3	77.67±1.45	90.33±0.88	60.33±1.20	34±1.15	26.67±0.88
4	67.67±1.86	85.33±0.88	51.67±0.67	28±1.15	20.67±1.86
5	56.33±1.86	73.33±1.45	45.33±0.88	25.33±0.88	18±1.53
6	48.67±1.20	66.33±1.45	40.33±0.33	23±0.58	16.67±0.88
7	39.33±1.45	47.33±1.20	38±0.58	20±1.00	14.33±0.88
8	34.67±0.88	41±1.15	33.33±0.33	17.33±1.76	13.00±1.53
9	32±1.15	34.33±1.76	29±1.15	14.33±1.20	11.33±0.88
10	26±1.16	28.33±0.67	25.67±1.76	11.33±0.88	8.33±0.88
11	23.33±1.20	23.67±0.88	18.67±2.01	11±1.15	5±0.58
12	25±1.15	26.67±1.45	21±1.73	12.67±0.88	6.33±0.88
13	17.67±1.20	20.67±0.88	13.33±1.20	10.33±0.33	4±0.58
14	14±0.58	18.33±0.88	10.67±0.88	8±1.73	3.33±0.67
15	12±1.15	15.33±0.88	8.67±0.67	7±1.15	2±0.58
16	11±1.15	12.33±1.33	7.33±0.88	6±0.58	0.67±0.33
17	8.33±0.88	10.67±1.20	5.33±0.88	5.67±0.33	0±0
18	6.33±0.88	8±0.58	3.67±0.33	3.67±1.33	0±0
19	2±0.58	4±0.58	2±1.15	1.33±0.88	0±0
20	0±0	0±0	0±0	0±0	0±0
21	0±0	0±0	0±0	0±0	0±0
Total	659.33	768	601	328	218.33

Appendix Table 2. Daily mean biogas yields from thermal pre-treatment test ± SE (mL) (n=3)

	Thermal Pre-treatment		
Days	Control	60 ºC	80 ºC
1	100±1.15	99.67±0.88	107.67±1.45
2	97±1.15	96.33±0.33	104.33±0.88
3	90.33±0.88	93±1.53	102.67±1.20
4	85.33±0.88	90.33±1.20	99.33±0.67
5	73.33±1.45	82.67±1.45	95±1.15
6	66.33±1.45	87±1.53	96±0.58
7	47.33±1.20	72.67±0.67	83.33±0.67
8	41±1.15	76.33±0.67	88.33±0.88
9	34.33±1.76	51±1.15	70.67±0.88
10	28.33±0.67	44±1.73	53.33±1.20
11	23.67±0.88	36.33±1.45	46±1.15
12	26.67±1.45	25±0.58	37.67±1.45
13	20.67±0.88	19.33±0.88	30.33±1.33
14	18.33±0.88	15±0.58	24.33±1.20
15	15.33±0.88	11.33±0.88	22.67±1.45
16	12.33±1.33	6±0.58	19±1.16

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17	10.67±1.20	3±0.58	11±1.15
18	8±0.58	0±0	0±0
19	4±0.58	0±0	0±0
20	0±0	0±0	0±0
21	0±0	0±0	0±0
Total	768	909	1091.67

Appendix Table 3. Daily mean biogas yield from CaO pre-treatment test ± SE (ml) (n=3)

	Chemical pre-treatment (CaO)			
Days	Control	0.5 g	1.5 g	2.5 g
1	100±1.15	0±0	0±0	0±0
2	97±1.15	12.33±1.45	0±0	0±0
3	90.33±0.88	90.67±1.20	62.67±1.45	17.67±1.45
4	85.33±0.88	103.33±0.88	117±1.15	121.67±1.20
5	73.33±1.45	93.66±1.45	99.67±1.76	115±1.73
6	66.33±1.45	86.67±1.20	89.67±0.88	108.33±0.88
7	47.33±1.20	75±1.00	79±1.15	99±1.15
8	41±1.15	71±1.15	72±1.00	92±1.15
9	34.33±1.76	75.33±1.45	78±1.52	86.67±0.88
10	28.33±0.67	51.33±0.67	64.33±0.88	74±2.08
11	23.67±0.88	45.33±1.76	59.33±1.45	64.33±1.20
12	26.67±1.45	40±0.58	51±0.58	62±1.00
13	20.67±0.88	35.33±0.88	53.33±0.88	64±1.15
14	18.33±0.88	27.33±1.45	43±1.16	53.67±1.45
15	15.33±0.88	23.33±1.20	38.67±0.88	48.67±0.88
16	12.33±1.33	15.33±1.33	33±1.15	42.67±1.20
17	10.67±1.20	10±1.15	24±1.53	36.67±1.20
18	8±0.58	5.67±1.20	14.67±1.45	26±1.53
19	4±0.58	2.33±0.88	5.67±1.76	11.67±1.20
20	0±0	0±0	0±0	0±0
21	0±0	0±0	0±0	0±0
Total	768	874	985	1124

Appendix Table 4. ANOVA: Single Factor for co-digestion.

Source	DF	Squares	Mean Square	F Value	Pr> F
Model	20	51153.0794	2557.65397	671.38	<.0001
Error	42	160	3.8095		
Corrected Total	62	51313.0794			

%CV=5.99, LSD=3.2161

Appendix Table 5. Fisher's least significant difference test for co-digestion

t Grouping	Mean	N	trt

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A	328	3	75% OP+25% PM
A			
A	601	3	50% PM+50% OP
B	768	3	75% PM+25% OP
B			
B	659.33	3	100% PM
C	218.33	3	100% OP

N.B. Means with the same letter are not significantly different.

Appendix Table 6. ANOVA: Single Factor for thermal pre-treatment.

Source	DF	Squares	Mean Square	F Value	Pr> F
Model	20	96995.2698	4849.7635	1520.08	<0.0001
Error	42	134	3.1905		
Corrected Total	62	97129.2698			

%CV=3.42, LSD=2.94

Appendix Table 7. Fisher's Least Significant Difference test for thermal pre-treatment

t Grouping	***Mean***	***N***	***trt***
A	***1098.33***	***3***	***80 ºC***
A			
A	***913.33***	***3***	***60 ºC***
B	***768***	***3***	***control***

N.B. Means with the same letter are not significantly different.

Appendix Table 8. ANOVA: Single Factor for NaOH pre-treatment.

Source	***DF***	***Squares***	***Mean Square***	***F Value***	***Pr> F***
Model	*20*	*75994.38095*	*3799.71905*	*1027.39*	*<0.0001*
Error	*42*	*155.33333*	*3.69841*		
Corrected Total	*62*	*76149.71429*			

%CV=4.67, LSD= 3.17

Appendix Table 9. Fisher's least Significant Difference test for CaO pre-treatment

t Grouping	Mean	N	trt
A	1124	3	2.5
B	985	3	1.5
C	874	3	0.5
C	768	3	Control

N.B. Means with the same letter are not significantly different.